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Søndergaard, Mette Sofie Rousing; Löfström, Charlotta; Al-Habib, Zahra Fares Sayer; Nielsen, M.T.; Hoorfar, Jeffrey; Josefsen, Mathilde Hasseldam

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Improved detection of *Salmonella* and *Campylobacter* through the optimized use of DNA polymerases in diagnostic real-time PCR

Mette S.R. Søndergaard, C. Löfström, Z.F.S. Al-Habib, M.T. Nielsen, J. Hoorfar and M.H. Josefsen

National Food Institute, Technical University of Denmark.

Aim
To investigate the performance of different DNA polymerases and master mixes in real-time PCR and their resistance towards inhibitors in matrices relevant for food safety, using validated PCR assays for *Salmonella* and *Campylobacter*.

Introduction
To improve food safety it is important to pursue fast, well performing and low-cost methods for detection of foodborne pathogens. Though real-time PCR offers several advantages compared with classical microbiology, the choice of a suitable DNA polymerase has been shown to optimize method performance considerably[1].

Screening:

Table 1. 16 DNA polymerases and 4 master mixes screened in standardized *Salmonella* and *Campylobacter* qPCR assays[2,3] on a 10-fold dilution series of purified DNA, ranked after performance.

DNA Polymerase or Master mix	Performance on *Salmonella*			Performance on *Campylobacter*			Con-clusion	Price [USD/U]
	Rating	LoD [µg/ml]	Max dR	Rating	LoD [µg/ml]	Max dR		
Tth DNA Polymerase (Roche)	+++	1.2×10^{-6}	42602	+++	6.6×10^{-7}	48971	+++	1.22
VeriQuest™ Probe qPCR Master Mix (Affymetrix)	+++	$1.2 \times 10^{-5*}$	38063	+++	6.6×10^{-7}	43067	+++	0.92
AmpliTaq Gold® (Applied Biosystems)	+++	1.2×10^{-6}	34546	++	6.6×10^{-7}	28147	+++	0.75
HotMaster® Taq DNA Polymerase (5 Prime)	+++	$1.2 \times 10^{-5*}$	39244	++	6.6×10^{-7}	16543	+++	0.66
TaKaRa Ex Taq® Hot Start Version (TaKaRa Bio Inc)	+++	$1.2 \times 10^{-5*}$	37222	++	6.6×10^{-7}	24549	+++	1.04
AmpliTaq® DNA Polymerase (Applied Biosystems)	++	$1.2 \times 10^{-5*}$	40053	++	6.6×10^{-7}	24930	++	0.80
AmpliTaq® 360 DNA Polymerase (Applied Biosystems)	++	$1.2 \times 10^{-5*}$	39484	++	6.6×10^{-7}	35547	++	0.56
AmpliTaq Gold® 360 DNA Polymerase (Applied Biosystems)	+++	1.2×10^{-5}	41110	++	6.6×10^{-7}	28090	++	1.29
TaqMan® Fast Advanced Master Mix (Applied Biosystems)	++	1.2×10^{-6}	34546	++	6.6×10^{-6}	28331	++	1.20
SG qPCR Master Mix (EURx)	+++	1.2×10^{-6}	30894	+	6.6×10^{-6}	6996	IC	0.46
HotStarTaq® Master Mix kit (Quiagen)	+++	$1.2 \times 10^{-5*}$	37751	+	6.6×10^{-4}	14648	IC	0.99
PicoMaxx High Fidelity PCR System (Agilent Technologies)	+	1.2×10^{-3}	24797	++	6.6×10^{-6}	19700	IC	1.00
FastStart Taq DNA Polymerase (Roche)	+++	$1.2 \times 10^{-5*}$	39644	-	6.6×10^{-2}	5091	IC	1.07
MyTaq™ HS DNA polymerase (Bioline)	+	1.2×10^{-5}	5448	+	6.6×10^{-7}	5338	+	0.66
MyTaq™ DNA Polymerase (Bioline)	+	1.2×10^{-5}	5126	+	6.6×10^{-6}	5733	+	0.33
Titanium™ Taq DNA Polymerase (Clontech)	-	1.2×10^{-5}	688	+ (72°C)	6.6×10^{-6}	8584	-	2.68
OneTaq® DNA Polymerase (New England BioLabs)	-	ND		+	6.6×10^{-5}	12860	-	0.20
Phusion® High-Fidelity DNA Polymerase with GC buffer (New England BioLabs)	-	1.2×10^{-5}	2676	-	ND		-	0.84
Pfu DNA Polymerase (Fermentas)	-	1.2	2358	-	ND		-	0.68
Herculase II Fusion DNA Polymerase (Agilent Technologies)	-	ND		-	ND		-	0.07

+++ Very good, ++ Good, + Intermediate, - Poor, ND not detected, IC inconclusive, *LoD could be lower.

Further evaluation of top 5:

Table 2. The performance of the four polymerases and the master mix, with the best results in the screening, on three different DNA extractions methods on:
A. Meat artificially contaminated with *Salmonella* and
B. Feces artificially contaminated with *Campylobacter*.

A.	Magnetic beads based DNA extraction			Lysis by boiling			Non-extracted		
DNA Polymerase or master mix	Rating	LoD [CFU/ml]	Max dR	Rating	LoD [CFU/ml]	Max dR	Rating	LoD [CFU/ml]	Max dR
Tth	++	10^{2*}	59615	+	10^{3}	47634	+	10^{5}	11710
VeriQuest MM	++	10^{2*}	28736	++	10^{2}	17216	++	10^{4}	7430
AmpliTaq Gold	++	10^{2*}	64637	++	10^{2*}	147690	++	10^{4}	12874
HotMaster Taq	++	10^{2}	35691	+++	10^{2*}	97583	++	10^{4}	23048
TaKaRa ExTaq HS	-	10^{3}	27058	-	10^{4}	47569	-	ND	257

B.	Qiagen kit extraction			Magnetic beads based DNA extraction			Lysis by boiling		
DNA Polymerase or master mix	Rating	LoD [CFU/ml]	Max dR	Rating	LoD [CFU/ml]	Max dR	Rating	LoD [CFU/ml]	Max dR
Tth	++	10^{3}	22022	-	NA		-	10^{6}	SD
VeriQuest MM	++	10^{2}	12661	-	NA		+	10^{4}	5578
AmpliTaq Gold	+	10^{3}	6643	-	NA		-	NA	
HotMaster Taq	+	10^{3}	9088	-	NA		-	10^{6}	SD
TaKaRa ExTaq HS	++	10^{2}	12661	+	10^{3}	7861	-	10^{6}	SD

+++ Very good, ++ Good, + Intermediate, - Poor, NA no amplification, SD single detection, *LoD could be lower, but only 10^2 to 10^6 CFU/ml was tested

Decreasing purity of DNA extractions

Examples of good, intermediate, and poor performance on high to low DNA purity:

Figure 1. Performance on magnetic beads-based extracted meat samples

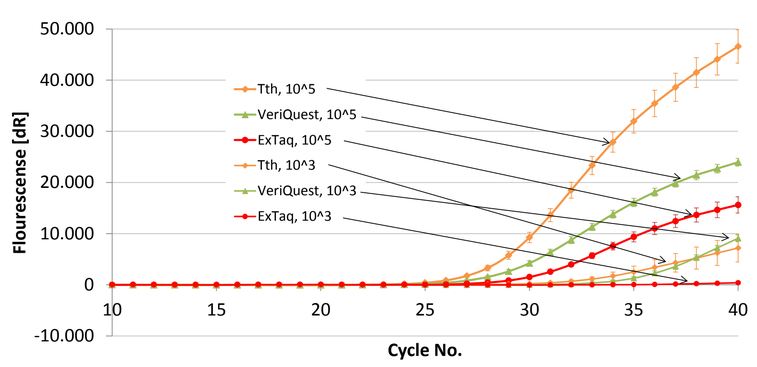


Figure 2. Performance on lysis by boiling extracted meat samples

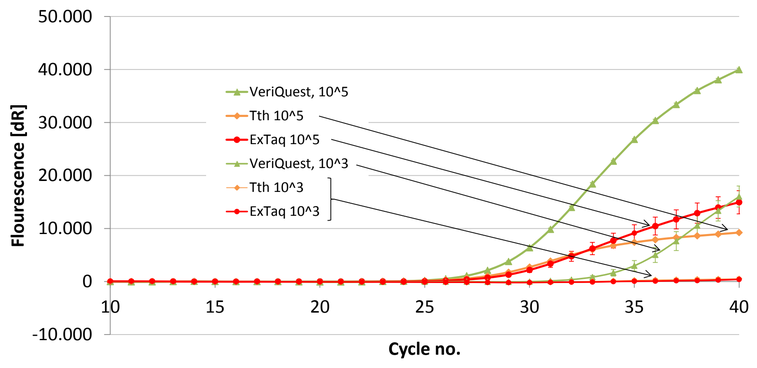
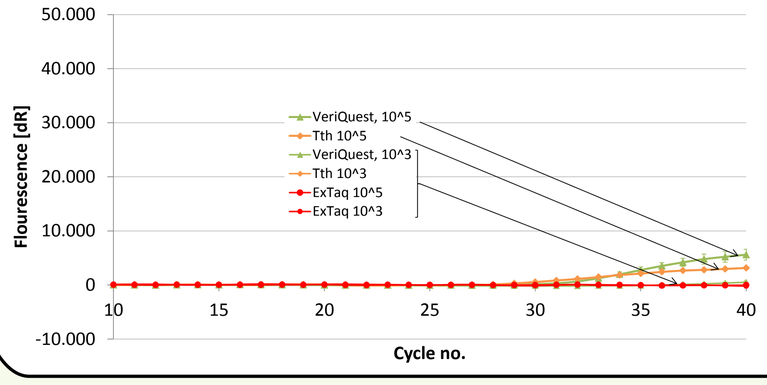


Figure 3. Performance on non-extracted meat samples



Decreasing purity of DNA extractions

Materials and Methods
16 commercially available DNA polymerases and 4 master mixes were included (see Table 1)
These were evaluated on a dilution series of purified *Salmonella* ser. Typhimurium and *Campylobacter jejuni* DNA, analyzed by standardized real-time PCR assays[2,3] using the accompanying PCR buffers for each polymerase.

The 5 best performing polymerases/kits were further evaluated using minced pork meat samples (diluted in BPW 1:10 and enriched for 18 h at 37°C followed by artificial contamination with *Salmonella* ser. Typhimurium ,10^2-10^6 CFU/ml) and chicken feces samples (artificially contaminated with *Campylobacter jejuni,* 10^2-10^6 CFU/ml). DNA extraction was performed on the samples by three different methods (Magnetic beads-based (KingFisher), lysis by boiling, and non-extracted for *Salmonella* and QIAamp® Fast DNA Stool Mini Kit (Qiagen), magnetic beads-based, and lysis by boiling for *Campylobacter*) followed by real-time PCR.
Polymerases were rated based on shape of amplification curves, amplification efficiency (AE), linear range and linearity of standard curve (R^2) and max flourescence (Max dR)

Conclusions
- The performances of the tested DNA polymerases varied considerably, reinforcing the importance of careful selection of an appropriate DNA polymerase for the PCR assay and sample type in question.
- For *Salmonella* in minced meat samples, HotMaster Taq, AmpliTaq Gold and VeriQuest were found to be the best performing alternative DNA polymerases.
- For *Campylobacter* in chicken feces samples, VeriQuest and ExTaq were found to be the best performing alternative DNA polymerases.

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3. Josefsen et al (2010) AEM, 76(15):5097.

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Mette Sofie Rousing Søndergaard
PhD Student
National Food Institute, DTU
Division of Food Microbiology
msrso@food.dtu.dk